

Epidemiological tracing of *Salmonella enterica* serotype Abortusovis from Spanish ovine flocks by PFGE fingerprinting

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SUMMARY

Salmonella enterica serotype Abortusovis, an ovine host-specific serotype, rare in most countries, is responsible for epidemic abortion episodes in Spain. With the aim of surveillance and detection of the spread of specific clones, 55 Abortusovis isolates collected during 1996–2001 from flocks in 11 provinces, were studied using *Xba*I–PFGE. Despite the fact that the strains were geographically and spatially related, PFGE demonstrated an epidemiologically acceptable discriminating power, identifying 20 clones (similarity, 52–96%). Clones Sabv6, 1, 5 were disseminated in seven, five and two areas respectively, while another 17 clones appeared in single places. Clones from nearby geographic regions showed a high relatedness (one band of difference in the PFGE profile) Sabv1-2-3, Sabv5-6, Sabv7-8, and Sabv13-14, suggesting a common ancestor. Co-isolation in the same flock (Sabv5-6, Sabv1-3, Sabv1-6) was detected. PFGE surveillance detected the predominance and widespread distribution of clone Sabv6 in 21 out of the 55 Abortusovis serotype episodes studied in Spain.

INTRODUCTION

Salmonella enterica subsp. *enterica* ser. Abortusovis is an ovine host-specific serotype, and is the most frequent cause of abortion outbreaks, stillbirths, and illness in lambs infected at birth in ovine flocks [1]. Available epidemiological data indicated that Abortusovis serotype is rare in most countries and regions of the world except in Europe, where it is particularly common, with reported cases in France, Spain, Germany, Cyprus, Italy, Switzerland, Russia,

and Bulgaria, southwest England and Wales and also in Western Asia [2, 3].

The infection is introduced into flocks by means of animal carriers such as new sheep replacements, contact with other animals in seasonal migration, wild and carrion birds, rodents [4]. The oral route is the main route of infection, from food and water contaminated by vaginal discharges, placenta, aborted fetuses (liver and stomach contents), and infected newborn. Furthermore, in some conditions, faeces, milk and respiratory secretions can correspond to infectious material. Other routes of acquisition include respiratory and conjunctival routes [5]. Of minor importance, but also reported, is venereal spread from contact with a contaminated sheep, the male ovine introduces the infection into the flock [3].

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From the third month of pregnancy, this pathogen induces abortion, in the absence of other clinical symptoms [5], but sometimes preceded by depression, uncertain walking, mucous vaginal discharge and diarrhoea. Following this, ewes seemed to be healthy or showed transient fever [6]. Sometimes, ewe mortality occurs from septicaemic complications and anorexia, acute metritis, enteritidis and peritonitis resulting from placental retention (5–7% of cases) [7], differing from infection causes by Dublin and Typhimurium serotypes. In addition, neonatal mortality of lambs is frequent with living lambs at term which are non-viable and die within a few hours of birth from septicaemia. Occasionally, lambs appear to be healthy but die during the first month, showing signs of enteritis, pneumonia or polyarthritis. Conversely, the infection is asymptomatic in non-pregnant ewes and in rams [3, 6].

Few epidemiological surveys have been able to be carried out owing to the low number of available *Abortusovis* isolates. Ribotyping, plasmid profiling, and IS200 fingerprinting [8–11], were performed as molecular characterization techniques with success to identify different and predominant *Abortusovis* genotypes.

To date, the highly discriminatory method of pulsed-field gel electrophoresis (PFGE) has not been used as a genotypic marker in *Abortusovis* serotype. The aim of this study was to survey and to detect the spread of specific clones causing epidemic abortions in ewe flocks at different geographical areas in Spain during a 5-year period by using PFGE typing. Susceptibility testing was also done, to provide insight into the antimicrobial resistance of this serotype in Spain.

MATERIAL AND METHODS

Bacterial strains

Fifty-five field strains of *Salmonella enterica* serotype *Abortusovis*, collected from epidemic abortions or neonatal mortality episodes affecting different ovine flocks from Spanish provinces during the 1996–2001 period (Table, Fig. 1), were submitted to the *Salmonella* and *Shigella* Spanish National Reference Laboratory (LNRSSE), for serotype confirmation. Strains were isolated from pathological samples such as vaginal discharges ($n=17$), placentas and aborted foetus or dead lambs' tissues (liver and stomach contents) ($n=38$). Biochemical identification was

performed by conventional methods and serotyping by slide agglutination using commercial *Salmonella* antisera (Staten Serum Institut, Copenhagen, Denmark). *Escherichia coli* ATCC 25922 was used as a control strain in the disk diffusion study, while the *S. enterica* serotype Braenderup reference strain was used as a control strain included in each gel of the PFGE as a global reference pattern (as in Salm-gene) [12].

Antimicrobial susceptibility testing

All strains were screened for resistance to 18 antimicrobial agents by the disk diffusion method according to CLSI guidelines [13] on Mueller–Hinton agar (BD Diagnostic Systems, Heidelberg, Germany). The antimicrobial agents tested were purchased by Oxoid (Unipath, Basingstoke, Hants., UK) and were as follows: ampicillin (10 µg/disk), amoxicillin-clavulanate (30 µg/disk), ticarcillin (75 µg/disk), cephalothin (30 µg/disk), cefotaxime (30 µg/disk), spectinomycin (Sh, 10 µg/disk), streptomycin (S, 10 µg/disk), gentamicin (10 µg/disk), tobramycin (10 µg/disk), apramycin (15 µg/disk), nalidixic acid (30 µg/disk), ciprofloxacin (5 µg/disk), enrofloxacin (5 µg/disk), tetracycline (30 µg/disk), sulphamethoxazole (Su, 300 µg/disk), trimethoprim–sulphamethoxazole (SXT, 1.25 and 23.75 µg/disk), chloramphenicol (30 µg/disk), and polymixin B (500 µg/disk). In addition, trimethoprim–sulphamethoxazole resistance was confirmed by the E-test (AB Biodisk, Solna, Sweden). CLSI standard break-points for Gram-negative enteric organisms were used as interpretative criteria, and intermediate categories were considered as resistant [13].

IS200 detection by PCR

Genomic DNA extractions of all strains for PCR assay were obtained by the 'boiling method' [14]. Primers and amplification conditions of DNA carrying an IS200 fragment were as previously described by Beuzón *et al.* [15]. Marker X (Roche Diagnostics, Barcelona, Spain) was used as a molecular weight marker.

PFGE and phylogenetic analysis

The PFGE technique was performed as outlined by the Salm-gene protocol [12] by using the CHEF-DRII System (Bio-Rad Laboratories, Hercules, CA, USA)

Table. Epidemiological characteristics of 55 *Abortusovis* strains isolated during a 5-year period in Spanish ovine flocks

Strain no.	Clinical origin	Date of Isolation (d/m/y)	Location (province, village)	PFGE profile	Resistance profile	Strain no.	Clinical origin	Date of Isolation (d/m/y)	Location (province, village)	PFGE profile	Resistance profile
989/96	Foetus	25.2.96	Bilbao	Sabv13	SShSu	3374/00	Abortus	31.3.00	Zamora	Sabv5	SSh
374/97	Placenta	23.1.97	Bilbao	Sabv14	SShSu	8370/00	Abortus	1.7.00	Zamora	Sabv5	SSh
857/97	Foetus	15.1.97	Bilbao	Sabv14	SShSuSXT	15/98	Liver	20.12.97	Ciudad Real (V. rubia)	Sabv6	SSh
5582/97	Foetus	6.11.97	Bilbao	Sabv16	SShSu	18/98	Liver	10.1.98	Ciudad Real (V. rubia)	Sabv1	SShu
320/98	Foetus	16.1.98	Bilbao	Sabv17	SShSu	19/98	Stomach	14.1.98	Ciudad Real (V. rubia)	Sabv1	SSh
1032/98	Foetus	15.3.98	Bilbao	Sabv18	SSh	1456/00	Liver-lung	1.2.00	Cuenca	Sabv11	SShSu
5083/98	Foetus	22.10–98	Bilbao	Sabv18	SShSu	3391/00	Abortus	14.4.00	Cuenca	Sabv6	SSh
6968/98	Foetus	13.11.98	Bilbao	Sabv17	SSh	24/98	Liver	29.1.98	Badajoz (Castuera)	Sabv1	SShSuSXT
1489/01	Foetus	28.2.01	Bilbao	Sabv14	SShSu	43/99	Liver	14.1.99	Badajoz (Castuera)	Sabv2	SSh
1490/01	Foetus	6.3.01	Bilbao	Sabv14	SShSu	1/97	Liver	12.1.97	Jaén (S. Espada)	Sabv1	SShSuSXT
4966/00	Vaginal d.	13.1.00	Huesca (P. Morena)	Sabv6	SSh	2/97	Liver	10.3.97	Jaén (S. Espada)	Sabv6	SSh
4967/00	Vaginal d.	20.1.00	Huesca (Robres)	Sabv19	SSh	4/97	Liver	14.4.97	Jaén (S. Espada)	Sabv6	SShSu
4968/00	Vaginal d.	24.1.00	Huesca (San Juan)	Sabv6	SShSu	5/97	Liver	14.4.97	Jaén (S. Espada)	Sabv5	SSh
4975/00	Vaginal d.	3.3.00	Huesca (Sariñena)	Sabv20	SSh	7/97	Liver	30.9.97	Jaén (S. Espada)	Sabv6	SSh
4977/00	Vaginal d.	24.3.00	Huesca (Senegues)	Sabv6	SSh	8/97	Stomach	14.10.97	Jaén (S. Espada)	Sabv4	SSh
4980/00	Vaginal d.	13.4.00	Huesca (La Pau)	Sabv6	SSh	9/97	Liver	17.12.97	Jaén (S. Espada)	Sabv6	SSh
770/99	Foetus	1.2.99	Zaragoza	Sabv12	SShSu	10/97	Stomach	17.12.97	Jaén (S. Espada)	Sabv3	SShu
772/99	Foetus	1.2.99	Zaragoza	Sabv15	SShSu	11/97	Stomach	17.12.97	Jaén (S. Espada)	Sabv1	SShu
4970/00	Vaginal d.	31.1.00	Zaragoza (V. Mayor)	Sabv6	SSh	12/97	Liver	19.12.97	Jaén (S. Espada)	Sabv6	SSh
4971/00	Vaginal d.	31.1.00	Zaragoza (V. Mayor)	Sabv6	SSh	13/97	Stomach	19.12.97	Jaén (S. Espada)	Sabv6	SSh
4972/00	Vaginal d.	31.1.00	Zaragoza (V. Mayor)	Sabv6	SSh	20/98	Stomach	20.1.98	Jaén (S. Espada)	Sabv10	SShSu
4973/00	Vaginal d.	31.1.00	Zaragoza (Figuerelas)	Sabv9	SSh	23/98	Stomach	20.1.98	Jaén (S. Espada)	Sabv10	SSh
4974/00	Vaginal d.	17.2.00	Zaragoza (Torres)	Sabv6	SSh	29/98	Liver	12.2.98	Jaén (S. Espada)	Sabv6	SSh
4978/00	Vaginal d.	22.3.00	Zaragoza (Traste)	Sabv6	SSh	30/98	Stomach	12.2.98	Jaén (S. Espada)	Sabv1	SSh
4979/00	Vaginal d.	10.4.00	Zaragoza (Monegrillo)	Sabv6	SSh	6/97	Liver	18.4.97	Córdoba	Sabv6	SShSuSXT
4981/00	Vaginal d.	2.5.00	Zaragoza (A. Negra)	Sabv8	SSh	42/99	Stomach	15.1.99	Córdoba	Sabv1	SSh
4982/00	Lung	2.5.00	Zaragoza	Sabv1	SShSu	14/98	Liver	09.1.98	Sevilla (Osuna)	Sabv6	SShSu
4977/00	Vaginal d.	22.3.00	Teruel	Sabv7	SShSu						

Vaginal d., vaginal discharge; S, streptomycin; Sh, spectinomycin; Su, sulphamethoxazole; SXT: trimethoprim–sulphamethoxazole.



Fig. 1. Map of Spain indicating the geographic origin of the epidemic episodes of *Abortusovis* serotype in ovine flocks, the different clones identified in each area (Sabv), and the number of isolates collected of each clone.

in 0.5× Tris–borate–EDTA at 14 °C and a 6 V/cm gradient with pulse ramping from 2 to 64 s over 22 h. *Xba*I-digested fragments were visually compared and interpreted in accordance with established criteria [16]. Only strains with an identical profile were considered to represent a single clone.

To analyse PFGE profiles, computer-monitored fingerprinting was performed with Gel Compar (Bio-Rad, Hemel Hempstead, Herts., UK) with TIFF files. Band patterns were scored for the presence and absence of bands at given molecular weights, and strains that differed by one band were assigned different PFGE profiles that were encoded by a number preceded by ‘Sabv’ (*Salmonella* Abortusovis serotype), after approval by HPA (Health Protection Agency, Colindale, UK). Cluster analyses using the Dice coefficient [17] for band matching with 0.8% tolerance position and a hierarchic unweighted pair-group method with averaging algorithm (UPGMA) were used to generate a dendrogram describing the

relationship among *Abortusovis* pulse types. The discrimination index was determined applying Simpson’s diversity coefficient [18].

RESULTS

Antimicrobial susceptibility testing

All strains showed full streptomycin and spectinomycin resistance, remaining susceptible to gentamicin and tobramycin. Although 23 out of 55 strains were sulphamethoxazole resistant, only four of them also expressed resistance to trimethoprim–sulphamethoxazole. When minimum inhibitory concentrations were determined by the E-test for this combination, the four strains, 857/97, 24/98, 1/97, and 6/97 showed a high level of resistance with values of 456:24, 456:24, 456:24, and >608:32 µg/ml. No β-lactams, quinolones, tetracycline or chloramphenicol resistance were observed in any of the studied strains. The

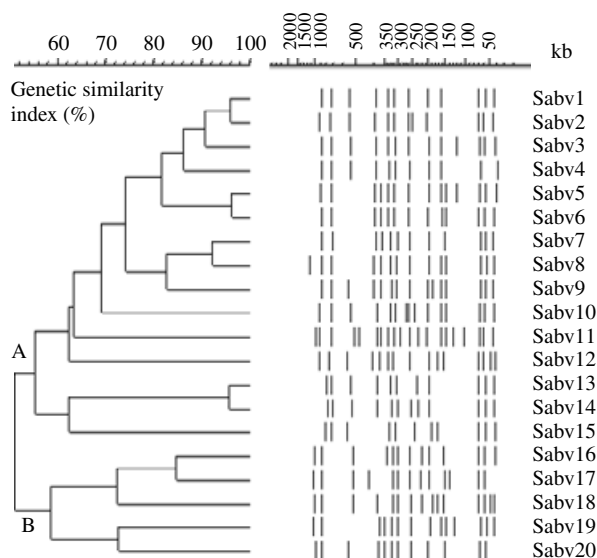


Fig. 2. Phylogenetic relationship among Spanish 55 isolates of *Salmonella enterica* serotype Abortusovis. The 20 identified pulsotypes are listed adjacent to the corresponding *Xba*I–PFGE banding pattern. Clustering (A and B) and subclustering (A1 and A2) were observed when an approximate 50% and 60% of genetic similarity coefficient was considered, respectively. DNA molecular-weight scale derived from Braenderup Salm-gene reference control.

antimicrobial resistance phenotypes found were: SSh phenotype (32 isolates), SShSu phenotype (19 isolates), and SShSuSXT phenotype (4 isolates). The epidemiological characteristics, and susceptibility profile of each strain, grouped by geographical origin, are shown in the Table.

IS200 detection

All strains resulted in an IS200 amplification DNA fragment of ~900 bp. Larger fragments were not identified in any of the Abortusovis strains.

PFGE and phylogenetic analysis

PFGE was used to characterize 55 *Salmonella* Abortusovis serotype strains recovered from ovine flocks. Under *Xba*I restriction, the number of bands originated for this serotype ranged from 11 to 20 (Fig. 2), with 20 different PFGE profiles (PFPs), Sabv1–20, as shown in the Table. Taking all isolates into account (55 related strains) Simpson’s diversity index reached a value of 0.865. Among the 20 PFPs, a wide genetic similarity coefficient value range was obtained, 52–96%. It was observed in the generated dendrogram (Fig. 2) that several of these PFPs showed

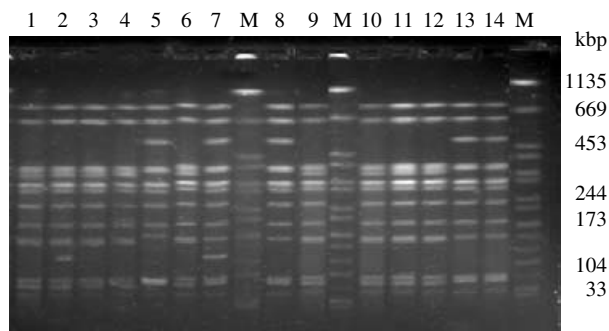


Fig. 3. PFGE profiling of Abortusovis strains. Lanes 1, 2, co-isolated strains no. 4/97 (Sabv6), 5/97 (Sabv5); lanes 3–5, 14/98 and 6/97 (Sabv6), 8/97 (Sabv4); lanes 6–8, co-isolated strains 9/97 (Sabv6), 10/97 (Sabv3), and 11/97 (Sabv1); lanes 9–11, 14, strains 15/98, 4966/00 and 4979/00 (Sabv6), 42/99 (Sabv1); lanes 12, 13, co-isolated strains 29/98 (Sabv6) and 30/98 (Sabv1). The control strain Braenderup serotype is used as molecular marker in lane M.

a higher genetic similarity (96%), with only one band difference, as occurs with Sabv1 and 2, Sabv5 and 6 (Fig. 3), and Sabv13 and 14. Differing also by one band, Sabv7 and 8, Sabv3 with respect to Sabv1 (Fig. 3), displayed 92% and 90% of relatedness respectively. Two clusters can be distinguished with nearly 50% similarity, a major cluster, termed A, which grouped the majority of the strains ($n=49$) and 15 PFPs (Sabv1–15), and the minor cluster B, with seven isolates and five PFPs (Sabv16–20). Some PFPs or clones, Sabv6, Sabv1, Sabv14, Sabv5, appeared at a higher frequency, and were observed in 21, seven, five, and four strains respectively (Fig. 3). Meanwhile 13 PFPs (23.6% of strains), were unique.

DISCUSSION

In contrast to other serotypes, such as Typhimurium and Enteritidis, that infect a wide range of hosts, causing a relatively mild enteric disease, some *Salmonella* serotypes are very host-restricted causing severe systemic disease, such as Typhi in humans and Abortusovis in sheep. The evolution of host specificity of *Salmonella* serotypes is linked with an increase in pathogenicity for the specific host [19]. Ovine salmonellosis may occur with a range of different symptoms of variable severity [5], depending mainly on the particular serotype involved, Abortusovis, Dublin and Typhimurium. The former is the main cause of ovine abortions in Europe and western Asia [4].

This serotype maintains the virulence for fetal and newborn lambs, and liver, spleen, brain and stomach

are the main sites of multiplication, with reduced virulence in adult sheep, evident during abortion [5]. By this route, the pathogen can disseminate in high numbers into the environment and infect new hosts [20]. The avoidance of an induced inflammatory response and mucosal damage by the highly host-restricted Abortusovis serotype may facilitate the systemic spread, invading the intestinal mucosa in low numbers [3]. The induction of abortion and the mortality of newborn lambs constitute a major economic problem in geographical areas with a sheep-based economy [21]. In endemic areas, this occurs in 30–60% of the ovine flock, mainly during the final stages of gestation of the first pregnancy, with a cyclic rhythm [4].

With the aim of tracing the heterogeneity of the Spanish clones of Abortusovis serotype, molecular subtyping with PFGE was performed on the 55 isolates collected from different ovine flocks with epidemic abortion from February 1996 to March 2001. This highly discriminatory method is a standard technique for the fingerprinting of *Salmonella* isolates and allowed us to analyse genetic relatedness and to identify the persistence and dissemination of any particular clonal type(s) of Abortusovis serotype in Spain.

To date, this is the first report where PFGE typing was applied in Abortusovis serotypes, with all the strains being typable. The discriminatory power of PFGE was evaluated using Simpson's diversity index (0.865) that expresses the probability that two unrelated strains will be placed in two different typing groups [18].

PFGE divided the Spanish Abortusovis strains into two clusters. Cluster A, which made up 89.1% of the strains can also be subdivided into two sub-clusters, A1 and A2. The most frequent PFPs were located within subcluster A1, with >60% similarity of the banding patterns, whereas 76.3% of isolates and 60% of PFPs were grouped. One clone from these clusters, Sabv6, was predominantly found among the Spanish strains studied (38.2%). It was detected for the first time in March 1997 in Santiago Espada (Jaén), and for last time in April 2000 in Monegrillo (Zaragoza).

While Sabv1, Sabv5, and Sabv6 clones were disseminated in five, two, and seven locations respectively, the other PFPs appeared in single places (Table, Fig. 1). However, there was no available information to show if these clones were responsible for outbreaks or if they were endemic strains. Moreover, 20 strains

with 16 PFPs emerged in a single episode in 11 areas, indicating that there were probably sporadic infections within the community.

Another event detected corresponded to the co-isolation of two clones in the same flock at the same time as occurred in Jaén, with Sabv 5 and 6, Sabv1 and 3, and Sabv1 and 6 (Fig. 3). This event had also been reported through IS200 fingerprints in two epidemic strains isolated in a single flock from northern Sardinia [10].

However, it is noteworthy that clones Sabv1, 5, 6, 14, 17 and 18, caused repeated infections in a specific area (Table). Hence, clone Sabv1 was identified 15 days and 1 year after the first detection in Ciudad Real and Jaén, respectively, while clone Sabv5 was also isolated 3 months later in Zamora. Similarly, clone Sabv6 was detected 4 months later in Huesca and Zaragoza, and 1 year later in Jaén (Fig. 3). Finally, in Bilbao, Sabv14, Sabv17 and Sabv18 were recovered 5 years, 11 months, and 8 months later, respectively, after first detection.

Factors which could possibly lead to the spread and increased prevalence of infection due to a specific clone of this organism corresponded to: a lack of food, which can contribute to growth of *Salmonella* in the rumen, changes in the digestive flora, intestinal and hepatic parasites, and individual factors associated with breed and genetic susceptibility. Other factors include animals meeting by seasonal migration or markets and the nomadic lifestyle of ovine flocks, insufficient health management and transport and a high proportion of pregnant ewes [1, 11].

Among the identified genotypes of the available Abortusovis serotype strains, it has been observed that closely related clones, with one band difference (90–96% similarity), emerged in the same geographic area: Sabv1 and 2, 1 year later in Badajoz; Sabv 1 and 3, nearly 1 year in Jaén; Sabv5 and 6, 5 months later in Jaén; and Sabv14 in Bilbao 1 year later; while in nearby places, e.g. Teruel and Zaragoza Sabv7 and 8 appeared. These data may suggest a common ancestor or a clonal evolution among strains yielded in a given area. An Italian survey of the presence of IS200 in Abortusovis strains from Sardinia also found that strains from nearby geographic regions showed high degrees of relatedness in their hybridization patterns, suggesting that four clones derive from a single chromosomal clonal line [10].

An epidemiological survey performed with 39 Iranian Abortusovis strains using IS200 fingerprinting, identified four genotypes in strains collected in

three provinces. Whereas 23 out of 34 strains from one province showed the same IS200 profile, as did three out of a total of three strains in a nearby province. This main genotype remained in that area from 1970 to the present day [11]. Furthermore, it was detected that the other three IS200 profiles of strains isolated in the west of Iran shared the four bands of the prevalent profile, plus an additional band. As stated by Nikbakht *et al.* [11] all these observations, suggest an ancestral clone which circulated throughout Iran for 30 years, and from which the other genotypes could be derived.

In the prevention of abortion caused by Abortusovis serotype, different antimicrobials such as streptomycin, tetracycline, chloramphenicol, furazolidone and more recently fluoroquinolones, have been used, although, results can be disappointing because some of these can induce endotoxemia [1]. Approximately 58.2% of our Abortusovis strains were susceptible to 16 out of the 18 antimicrobials tested. Resistance to streptomycin and spectinomycin were found in all strains, possibly as a consequence of overuse of these aminoglycosides in routine ovine practice in Spain. As a serotype, Abortusovis appears to be quite sensitive to β -lactams, quinolones, tetracycline, chloramphenicol, polymyxin B, and to other tested aminoglycosides. Conversely, as cited for other adapted serotypes (Typhimurium, Enteritidis, Hadar, Typhi), high levels of antimicrobial resistance in veterinary *Salmonella* strains as a consequence of antibiotic presence in feeds [22] has not been reported in Abortusovis [1, 23]. In contrast, resistance in Abortusovis strains to sulphamethoxazole was high (41.8%), while fewer isolates were trimethoprim-sulphamethoxazole resistant (7.3%), which is explained by the use of individual sulphonamides (or in association with tetracycline) as the most common antimicrobials used in the prophylactic treatment of numerous ovine pathologies (metritis, mastitis, diarrhoea, pneumonia, foot diseases or coccidiosis). However, antibiotic surveillance would be desirable as a consequence of affecting new antimicrobials, because ampicillin and tetracycline resistance has been previously reported in 10.3% and 3.4% of Italian Abortusovis strains recovered from 1997 to 2003 [24].

We did not detect a special gathering of clusters reflecting sulphamethoxazole and trimethoprim-sulphamethoxazole resistance, although, differences in susceptibilities were observed within clones Sabv1, 6, 14, and 18, either inside or outside the community.

In summary, fingerprinting of genomic DNA by *Xba*I–PFGE was particularly useful for defining clonal lines of Abortusovis strains and their spread in close geographic areas. It provided a good level of intra-serotype discrimination with zoonotic relevance which helps to detect the emergence of new strains and could be recommended as a typing method as has previously been used for other *Salmonella* spp. serotypes. During the 1996–2001 period, 20 clones of Abortusovis serotypes were identified in 11 Spanish provinces, and some events have been detected in this study: (i) most of the clones appeared in single places, although clones Sabv1, 5, and 6 were disseminated in five, two, and seven locations, respectively; (ii) 16 clones emerged in a single episode in specific areas, but some clones (Sabv1, 5, 6, 14, 17 and 18) caused repeated infections; (iii) several clones are co-detected in the same flock at the same time (Sabv5 and 6, Sabv1 and 3, Sabv1 and 6); (iv) clones Sabv6, 1, 14 and 5 were the predominant cause of the 38.2%, 12.7%, 9.1%, and 7.3% of studied Abortusovis episodes, respectively.

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DECLARATION OF INTEREST

None.

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